A1 Cont'd

domains for other IgG subtypes and domains from IgM, IgA,IgD and IgE antibodies. The modified fusion proteins can be expressed, purified and their specific activities determined in *in vitro* bioassays as described in the Examples.--

Please replace the paragraph on page 24, lines 27-34, with the following paragraph:

--To construct a di-peptide [ser-gly] linker, one can PCR the IgG1-Fc sequence with a 5' oligonucleotide that adds the 5' extension CGCTCCGGA to the hinge coding sequence. The TCCGGA hexanucleotide is a cleavage site for the restriction endonuclease *Bsp* EI and encodes amino acids ser-gly. This PCR fragment can be digested with *Bsp* EI and *Sac* II and the ~ 240 bp fragment cloned into similarly cut pCDN3.1(+)::EPO-IgG1-Fc and pCDNA3.1(+)::G-CSF-IgG1-Fc. The unique *Bsp* EI site in each of these plasmids occurs at the first ser-gly in the linker [ser-gly-gly-ser-gly-gly-ser] (SEQ ID NO:3) so that the resulting recombinants will contain this 2 amino acid, ser-gly, linker. The sequence of the newly inserted ~ 250 bp *Bsp* EI-*Sac* II fragment can be verified.--

A2

Please replace the paragraph spanning page 24, line 35 to page 25, line 3 with the following paragraph:

--A similar procedure can be used to construct the 4 amino acid [ser-gly-gly-ser] (SEQ ID NO:1) linker. One can PCR the IgG1-Fc sequence with a 5' oligonucleotide that adds the 5' extension CGCGGATCC to the hinge coding sequence. The GGATCC hexanucleotide is a cleavage site for the restriction endonuclease *Bam* HI and encodes amino acids gly-ser. This PCR fragment can be digested with *Bam* HI and *Sac* II and the ~ 240 bp fragment cloned into similarly cut pCDN3.1(+)::EPO-IgG1-Fc and pCDNA3.1(+)::G-CSF-IgG1-Fc. The unique *Bam* HI site in each of these plasmids occurs at the first gly-ser in the linker [ser-gly-gly-ser-gly-gly-ser] (SEQ ID NO:3) so the recombinants will contain the 4 amino acid (ser-gly-gly-ser) (SEQ ID NO:1) linker. The sequence of the inserted ~ 250 bp *Bam* HI – *Sac* II piece can be verified.--

A3

Please replace the paragraph on page 40, lines 1-10, with the following paragraph:

--In order to construct and express gene fusions of IFN-α2 with IgG coding sequences the IFN-α2 gene was modified at the 5' and 3' ends using PCR based mutagenesis. pBBT160 plasmid

A4

DNA was used as template for PCR with forward primer BB108 (5' CGCAAGCTTGCCACCATGGCCTTGACCTTT GCTTTA-3'; SEQ ID NO:46) and reverse primer BB109 (5'- CGCGGATCCTCCGGATTCCTTACTTCTTAAACTTTC-3'; SEQ ID NO:47). Primer BB108 anneals to the 5' end of the coding sequence for the IFN-α2 secretion signal and the reverse primer, BB109, anneals to the 3' end of the IFN-α2 coding sequence. The resulting PCR product was digested with *Hind* III and *Bam* HI, gel purified and cloned into pCDNA3.1(+) vector that had been digested with *Hind* III and *Bam* HI, alkaline phosphatase treated, and gel purified. A clone with the correct DNA sequence (Henco et al., 1985) was designated pCDNA3.1(+)::IFNAfus or pBBT190.--

Please replace the paragraph spanning page 51, line 32, to page 52, line 2, with the following paragraph:

--A GM-CSF-IgG1-Fc direct fusion can be created by PCR using plasmid pcDNA3.1::GM-CSF-IgG1-Fc as the DNA template. One PCR reaction can use oligos GMCSFDFA (5' GAGCCAGTCCAGGAGGAGCCCAAATCTTGTGACAAA-3'; SEQ ID NO:72) and BB82 (SEQ ID NO:14). The second PCR reaction can use oligos GMCSFDFB (5' ACAAGATTTGGGCTCCTCCTGGACTGGCTCCCAGCA –3; SEQ ID NO:73) and BB91 (SEQ ID NO:11). The products from these PCR reactions can be gel-purified, mixed and subjected to a third PCR reaction using oligos BB82 and BB91. The approximate 1150 bp PCR product can be gel-purified, digested with and *Hind* III and *Sac* II, the resulting ~ 675 bp *Hind* III/*Sac* II fragment gel-purified and cloned into similarly cut plasmid pcDNA3.1::GM-CSF-IgG1-Fc that had been treated with calf intestinal phosphatase. A clone with the correct insert can be identified by DNA sequencing.--

Please replace the paragraph on page 52, lines 3-12, with the following paragraph:

--A GM-CSF-IgG4-Fc direct fusion can be created by PCR using plasmid pcDNA3.1::GM-CSF-IgG4-Fc as the DNA template. One PCR reaction can use oligos GMCSFDFC (5'-GAGCCAGTCCAGGAGGAGTCCAAATATGGTCCCCCA-3')(SEQ.ID.NO.74) and BB82. The second PCR reaction can use oligos GMCSFDFD (5'-

ACCATATTTGGACTCCTCCTGGACTGGCTCCCAGCA-3')(SEQ.ID.NO.75) and BB91. The products from these PCR reactions can be gel-purified, mixed and subjected to a third PCR reaction using oligos BB82 and BB91. The approximate 1150 bp PCR product can be gel-purified, digested with and *Hind* III and *Sac* II, the ~ 675 bp *Hind* III/*Sac* II fragment gel-purified and cloned into similarly cut plasmid pcDNA3.1::GM-CSF-IgG4-Fc that has been treated with calf intestinal phosphatase. A clone with the correct insert can be identified by DNA sequencing.--

Please replace the paragraph spanning page 52, line 32, to page 53, line 11, with the following paragraph:

--**9. A. Cloning Stem Cell Factor (SCF):** SCF regulates development of hematopoietic progenitor cells. A cDNA encoding human SCF can be amplified by RT-PCR using RNA isolated from HepG2, 5637 or HT-1080 cell lines (Martin et al., 1990; the HepG2 and 5637 cell lines are available from the ATCC, Rockville, MD). PCR reactions can be carried out with forward primer SCF-F (5'- CGCAAGCTTGCCACCATGAAGAAGACACAAACT –3')(SEQ.ID.NO.76) and reverse primer SCF-R (5'-CGCGGATCCTCCGGAGTGTAGGCTGGAGTCTCCAGG –3')(SEQ.ID.NO.77). SCF DNA sequences in the primers are underlined. Primer SCF-F anneals to the 5' end of the coding sequence for the SCF secretion signal and the reverse primer, SCF-R, anneals to the 3' end of the SCF coding sequence, beginning at the junction of the extracellular and transmembrane domains. Other reverse PCR primers can be used to create truncated forms of the SCF extracellular domain, in particular a form that terminates following Ala-174 of the mature protein, by substituting appropriate nucleotides for the SCF DNA sequence listed in SCF-R. The resulting PCR product can be digested with *Hind* III and *Bam* HI, gel purified and cloned into pCDNA3.1(+) vector that has been digested with *Hind* III and *Bam* HI, alkaline phosphatase treated, and gel purified. Several clones can be sequenced to identify one with the correct DNA sequence. IgG1-Fc, IgG4-Fc, IgG1-C_H, IgG4-C_H and kappa light chain constant regions can be fused to the carboxy-terminus of the extracellular domain of SCF as described in Examples 1 and 5. The cell line TF-1 (Kitamura, 1989; available from the American Type Culture Collection, Rockville, MD) can be used to measure bioactivity of SCF-IgG fusion proteins.--

Please replace the paragraph on page 53, lines 12-22, with the following paragraph:

--Direct fusions of the extracellular domain of SCF to various IgG domains can be constructed using procedures similar to those described in Example 4 for constructing EPO-IgG and G-CSF-IgG direct fusions. A SCF-IgG1-Fc direct fusion can be created by PCR using plasmid pcDNA3.1::SCF-IgG1-Fc7AA as the DNA template. One PCR reaction can use oligos SCFDFF (5'-GACTCCAGCCTACACGAGCCCAAATCTTGTGACAAA-3')(SEQ.ID.NO.78) and BB82. The second PCR reaction used oligos SCFDFR (5'-ACAAGATTTGGGCTCGTGTAGGCTGGAGTCTCCAGG-3'; SEQ.ID.NO.79) and BB91. The products from these PCR reactions can be gel-purified, mixed and subjected to a third PCR reaction using oligos BB82 and BB91. The PCR product can be gel-purified, digested with and *Hind* III and *Sac* II, and cloned into similarly cut pcDNA3.1::SCF-IgG1-Fc7AA that had been treated with calf intestinal phosphatase. A clone with the correct insert can be identified by DNA sequencing.--

Please replace the paragraph on page 53, lines 23-31, with the following paragraph:

--A SCF-IgG4-Fc direct fusion can be created by PCR using plasmid pcDNA3.1::SCF-IgG4-Fc7AA as the DNA template. One PCR reaction can use oligos SCFDFC (5'-GACTCCAGCCTACACGAGTCCAAATATGGTCCCCCA-3')(SEQ.ID.NO.80) and BB82. The second PCR reaction can use oligos SCFDFD (5'-ACCATATTTGGACTCGTGTAGGCTGGAGTCTCCAGG-3') (SEQ.ID.NO.81) and BB91. The products from these PCR reactions can be gel-purified, mixed and subjected to a third PCR reaction using oligos BB82 and BB91. The PCR product can be gel-purified, digested with and *Hind* III and *Sac* II, and cloned into similarly cut pcDNA3.1::SCF-IgG4-Fc7AA that has been treated with calf intestinal phosphatase. A clone with the correct insert can be identified by DNA sequencing.--

Please replace the paragraph spanning page 53, line 33, to page 54, line 13, with the following paragraph:

--10. Flt-3L-IgG fusion Proteins: Flt-3L (Lyman et al., 1993; Hannum et al., 1994) is a membrane bound cytokine that regulates development of hematopoietic stem cells. A cDNA encoding human Flt-3L can be amplified by PCR from single-stranded cDNA prepared from adult

or fetal liver, kidney, heart, lung, or skeletal muscle, which is available from commercial sources such as CLONTECH and Stratagene, Inc.. PCR reactions can be carried out with forward primer fltF (5'- CGCAAGCTTGCCACCATGACAGTGCTGGCGCCAGCC –3') (SEQ.ID.NO.82) and reverse primer fltR (5'- CGCGGATCCTCCGGAAGGGGGCTGCGGGGCTGTCGG –3')(SEQ.ID.NO.83). Flt-3L DNA sequences in the primers are underlined. Primer fltF anneals to the 5' end of the coding sequence for the Flt-3L secretion signal and the reverse primer, fltR, anneals to the 3' end of the Flt3 coding sequence, beginning at the junction of the extracellular and transmembrane domains. Other reverse PCR primers can be used to create truncated forms of the Flt-3L extracellular domain by substituting appropriate nucleotides for the Flt-3L DNA sequence listed in primer fltR. The resulting PCR product can be digested with *Hind* III and *Bam* HI, gel purified and cloned into pCDNA3.1(+) vector that has been digested with *Hind* III and *Bam* HI, alkaline phosphatase treated, and gel purified. Several clones can be sequenced to identify one with the correct DNA sequence. IgG1-Fc, IgG4-Fc, IgG1-C_H, IgG4-C_H and kappa light chain constant regions can be fused to the carboxy-terminus of the extracellular domain of Flt-3L as described in Examples 1and 5. Ba/F3 cells transfected with the human Flt-3 receptor (Lyman et al.,1993; Hannum et al., 1994) can be can be used to measure bioactivity of Flt-3L-IgG fusion proteins. Ba/F3 cells are available from the German Collection of Microorganisms and Cell Cultures (Braunschweig, Germany).--

Please replace the paragraph on page 54, lines 14-24, with the following paragraph:

--Direct fusions of the extracellular domain of Flt-3L to various IgG domains can be constructed using procedures similar to those described in Example 4 for constructing EPO-IgG and G-CSF-IgG direct fusions. A Flt-3L-IgG1-Fc direct fusion can be created by PCR using the pcDNA3.1::Flt-3L-IgG1 plasmid described above as the DNA template. One PCR reaction can use oligos FltDFA (5'-GCCCCGCAGCCCCCTGAGCCCAAATCTTGTGACAAA-3'(SEQ.ID.NO.84) and BB82. The second PCR reaction can use oligos Flt3DFB (5'-ACAAGATTTGGGCTCAGGGGGCTGCGGGGCTGTCGG-3')(SEQ.ID.NO.85) and BB91. The products from these PCR reactions can be gel-purified, mixed and subjected to a third PCR reaction

using oligos BB82 and BB91. The PCR product can be gel-purified, digested with and *Hind* III and *Sac* II, and cloned into similarly cut pcDNA3.1::SCF-IgG1-Fc7AA that had been treated with calf intestinal phosphatase. A clone with the correct insert can be identified by DNA sequencing.--

Please replace the paragraph on page 54, lines 25-32, with the following paragraph:

--A Flt-3L-IgG4-Fc direct fusion can be created by PCR using plasmid pcDNA3.1::Flt3-IgG4-Fc7AA as the DNA template. One PCR reaction can use oligos FltDFC (5'-GCCCCGCAGCCCCCTGAGTCCAAATATGGTCCCCCA-3')(SEQ.ID.NO.86) and BB82. The second PCR reaction can use oligos FltDFD (5'-ACCATATTTGGACTCAGGGGGCTGCGGGGCTGTCGG-3')(SEQ.ID.NO.87) and BB91. The products from these PCR reactions can be gel-purified, mixed and subjected to a third PCR reaction using oligos BB82 and BB91. The PCR product can be gel-purified, digested with *Hind* III and *Sac* II, and cloned into similarly cut pcDNA3.1::SCF-IgG4-Fc7AA that has been treated with calf intestinal phosphatase. A clone with the correct insert can be identified by DNA sequencing.--

IN THE CLAIMS:

Please amend Claims 4-8, 12, 14, 17, 20-26, 28, 32, 34, 37, 38 and 42 as follows, without prejudice to or disclaimer of the subject matter therein. Claims 1-3, 9, 10, 15, 16, 18, 19, 27, 29-31, 33, 35, 36, and 39-41 are reiterated below, without amendment. Claims 43-61 have been added.

1. (Reiterated) A fusion protein comprising a soluble protein joined without an intervening peptide linker to an immunoglobulin (Ig) domain, wherein the soluble protein is selected from the group consisting of a growth factor, a cytokine that is not IL-10, and an active variant thereof, and wherein the immunoglobulin domain does not contain a variable region.

2. (Reiterated) A fusion protein comprising a soluble protein joined at its carboxy-terminus to the amino terminus of an immunoglobulin domain, wherein the soluble protein is selected from the group consisting of a growth factor, a cytokine that is not